Effect of different seed water content and storage duration on seed viability of local genotype brown rice Daro Merah

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Research Article

Effect of Different Seed Water Content and Storage Duration on Seed Viability of Local Genotype Brown Rice *Daro Merah*

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Abstract

Background and Objective: Most of rice seeds experience after ripening problem which then result in seed dormancy. This kind of dormancy could be broken through various types of treatments, including storaging in dry place for certain period. This study was aimed to evaluate the combination between seed water content and storage duration to induce the seed germination of local genotype brown rice, *Daro Merah*. **Materials and Methods:** Various levels of seed water content (6-10, 11-15, 16-20 and 21-25 %) and storage duration (2, 4, 6 and 8 weeks) were evaluated to identify its effect *on Daro Merah* seed germination. Data were analyzed using two-way analysis of variance where significance were further proceeded using Duncan's New Multiple Range Test with a $p<0.05$. **Results:** The results showed that seed germination of *Daro Merah* reached 95 % at five days after seeding when the seed water content was maintained at 6-10 % and stored for 8 weeks. After 14 days, the germination reached 99 % and most of the seeds showed some shoots emerged from the soil.

Key words: Brown rice, *Daro Merah*, germination, seed water content, storage duration.

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Introduction

Rice is the main food commodity considered as daily staple food which widely consumed in Indonesia. Due to society lifestyle who tends to be highly dependent in consuming rice, this crop always experiences very high demand along with the population growth rises. However, some people are getting more aware of their health recently and putting more attention in selecting the type of rice which provides both nutrition and health effect (Suardi, 2005). Of all rice types, brown rice are one of the choice for this circumstance.

Instead of being a staple food, brown rice had been widely known for its health benefits, such as for treating people suffered from vitamin A and B deficiencies as well as malnutrition. The anthocyanin content in this brown rice had been reported as a source of antioxidant which is beneficial to prevent various degenerative diseases, including coronary heart, cancer, diabetes, and hypertension (Suardi, 2005). Therefore, this kind of rice is promising to be further developed in order to fulfill the society demand on healthier rice type.

The utilization of brown rice had been carried out in several regions of West Sumatera, such as South Solok, West Pasaman, and Solok, eventhough its cultivation has not been as intensive as white rice. Several local genotypes of brown rice had been developed and one of them were *Daro Merah* originated from Batang Anai region. Unlike other local genotypes, Daro Merah is categorized as short duration genotype which mature around 115 days, thus enables farmers to harvest the yield earlier. However, the weakness of this local genotype comes from its seed characteristic. Similar to the most of rice seeds, Daro Merah seed also encounters dormancy problem due to after ripening effect. Generally, this kind of dormancy requires drying treatment for certain period to break the dormant stage (Sutopo, 2002). Due to this condition, the seed viability decreases significantly leading to growth failure when planted in the field.

After ripening problem is possibly solved by decreasing the seed water content through direct heating (using sunlight or oven) or dry storaging under room temperature. These two methods are regarded as a relatively easy and affordable approach to overcome this dormancy problem as well as applicable for farmers. Previous study proposed that the heating at 50°C for 48 hours followed by 48 hours soaking treatment using 3 % KNO_3 were possible to break the seed dormancy of all tested genotypes effectively (Wahyuni et al., 2004). This heat treatment was purposed to reduce the seed water content and accelerate the period of after ripening in rice seeds. According to this explanation, this study was conducted to evaluate the effect of seed water content and storage duration in triggering the dormancy break of Daro Merah seeds, thus increasing its seed viability.

Materials and Methods

The seeds of Daro Merah were collected after harvesting and were subjected into dry place storaging treatment for 2, 4, 6, and 8 weeks. Then seeds were heated at 50°C until reaching one of these four levels of water content, ranging from 6-10, 11-15, 16-20, and 21-25 %. Several seed tests were performed afterwards, including seed viability, first count, and soil emergence assays. These analysis were carried out by applying two factors (seed moisture content and storage duration) in block randomized design. The resulted data were analysed using two-way analysis of variance (anova) and significance was further determined using Duncan's New Multiple Range Test (DNMRT) with a $p<0.05$.

Results

Seed Water Content

The initial water content of Daro Merah seeds after being harvested was recorded more than 30%. This value might be associated with the harvesting activity executed under rainy condition leading to high absorption of water by the seeds. The reduction of water content into those four levels required various heating periods (Table 1). The highest level of seed water content (21-25 %) set in this study was obtained after 4 hours of drying. Longer drying period about 18 hours (overnight) was required to reduce the water content up to 6-10 %.

Table 1. Water content of Daro Merah seeds after heated at 50°C.

Range of expected water content (%)	Obtained seed water content (%)	Heating duration (hour)
21–25	21.3	4
16–20	20	6
11–15	12.8	14
6–10	9.9	18

Number of Normal and Abnormal Seedlings

Seed viability is an indicator to measure the seed ability to germinate into a normal or abnormal seedlings. For the seed users or consumers, viability provides the information regarding the seed quality and its possibility to produce normally under optimum biophysical environment. A normal seedling was characterized as a seedling with long primary roots complemented with numerous secondary roots and already emerged its first leaf (Fig. 1). As seen in Table 2, seed containing 6-10 and 11-15 % water contents stored for 8 weeks exhibited the highest percentage of normal seedlings. In contrast, the highest water content seeds (21-25 %) were unable to germinate, even after stored for 2 and 4 weeks (Table 2). Low percentage of normal seedlings were achieved after those seeds stored for 6 weeks and rose along with the increasing storage duration. Interestingly, the percentage of normal seedlings from all levels of water content reached maximum values after 8 weeks of storage (Table 2). It then suggested that the breaking of after ripening dormancy were greatly determined by the duration of storage. Additionally, it was also clearly seen that the less the water content followed by the longer the storage, thus leading to elevated percentage of normal seedlings emergence.

Unlike the normal seedlings, the appearence of abnormal seedlings resulted in this study was relatively very low. A seedling was considered as an abnormal seedling when it had no roots and first leaf or showed weak primary roots and plumules (Fig. 2). The highest percentage of abnormal seedlings found only reached 0.53 % from the driest rice seeds (Table 3). This result implied that the dormancy had been broken in few seeds, but the growth of the seedlings after that breaking was mostly considered as normal growth. In contrast, the wettest seeds revealed no abnormal seedlings formed (Table 3) due to the unbroken dormancy.

Number of Unviable and Dormant Seeds

Due to a strict pre-test selection, only small amount of unviable seeds were found during this study (Table 4). Prior to seed germination, the seeds were selected based on its uniformity, healthy, and purity indicating that it did not contaminated by any dirt or other unwanted seeds. The putative viable seeds were then separated from the improper seeds to minimize the percentage of unviable seeds germinated during the analysis. The unviable seeds found in this study were characterized as fungal-infected (Fig. 3a), darker-colored, and light-weighted seeds with softer texture if being pressed by hand. Commonly, an unviable seed would be immediately floating once it was soaked into water (Fig. 3b).

Table 2. Effect of seed water content and storage duration toward the number of germinated normal seedlings.

Expected water content (%)	Number of germinated normal seedlings (%) Storage duration (weeks) 2	4	6	8
21–25	49.33 aB	95.33 aA	90.00 aA	98.67 aA
16–20	20.67 Bc	78.00 Bb	96.00 Aa	98.67 aA
11–15	6.67 cC	68.67 bB	87.33 aA	93.33 aA
6–10	0.67 dB	0.00 cB	3.33 bB	12.67 bA
CV* = 7,56 %				

Numbers on the same row followed by the same uppercase and numbers on the same column followed by the same lowercase were insignificantly different according to DNMRT with a $p<0.05$.
*Coefficient of variance (CV) was obtained after the data transformed with $\sqrt{y+1}$.

Different with the unviable seeds, number of dormant seeds was mostly found under high seed water content condition with a short storage duration. Red rice seeds containing 21-25 % water exhibited 100% dormant seeds when stored for 4 weeks (Table 5). However, the best condition resulting in the least number of dormant seeds was achieved from the driest seeds stored for 8 weeks (Table 5). These results implied that the decreasing seed water content and the elongation of storage duration could minimize the occurence of dormant seeds.

Figure 1. Morphology of normal brown rice seedlings.

Seed Vigor

This parameter was determined through first count test to observe the speed of a seed to germinate within 5 days after seeding and its growth uniformity. This present study showed that the highest vigor was obtained from seed containing 6-10 % water stored for 8 weeks (Table 6). Shorter duration of storage applied on this level of water content still resulted in insignificantly different vigor values, except in the treatment of 2 weeks storage. In contrast, the highest water content seeds showed no germination after 2 and 8 weeks storage (Table 6). The germination of these seeds was recorded after 4 weeks storage about 14.67 %, but then strikingly dropped when the storage duration was prolonged up to 6 weeks (Table 6). According to these results, the speed of seed germination depended on the absence of dormancy problem. As the lowest water content and the longest storage duration applied, seeds enabled to completely break their dormancy and exhibited faster growth compared to other water content and storage duration treatments.

Table 3. Effect of seed water content and storage duration toward the number of abnormal seedlings.

Expected water content (%)	Number of abnormal seedlings (%) Storage duration (weeks)				Average
	2	4	6	8	
21–25	0.7	0.0	0.7	0.7	0.53
16–20	0.0	0.0	0.7	0.0	0.18
11–15	0.7	0.0	0.0	0.0	0.18
6–10	0.0	0.0	0.0	0.0	0.00
Average	0.35	0.00	0.35	0.18	
CV* = 22.10 %					

*Coefficient of variance (CV) was obtained after the data transformed with $\sqrt{y+1}$.

a b

Figure 2. Morphology of the abnormal brown rice seedlings marked by rotten plumule (a) or less and weak secondary root.

As shown in Fig. 4a, the germination of the driest seeds increased when the storage durations were prolonged. It implied that the after ripening dormancy would break when a seed encountered a very dry condition internal and externally for certain period. Unlike the lowest water content seeds, the germination pattern recorded from the highest water content seeds showed any depletion at 6 and 8 weeks storage. It might occur when the seeds unabled to response the environmental conditions created through the treatment application. This incapability was possibly related to the sign of seed deterioration that commonly happened, particularly due to the exposure of high water content for long period.

Soil Emergence

Soil emergence is also an indicator of seed vigor describing how strong the seed could grow during certain time period. In line with result of first count test (Table 6), the driest seeds showed the highest soil emergence after stored for 8 weeks (Table 7). The insignificantly different values was achieved from the shorter storage duration, except at 2 weeks storage. The lowest soil emergence was obtained from the seeds containing the highest water content after 8 weeks storage (Table 7). Interestingly, the soil emergence of this water content level increased after 4 weeks storage before rapidly decreased along with the increasing of storage duration (Fig. 4b).

a b

Figure 3. Appearance of unviable brown rice seeds showing the presence of fungal hyphae causing soft watery texture and rotten seed (a). Unviable seeds floated when being soaked on water due to volume loss (b).

Table 4. Effect of seed water content and storage duration toward the number of unviable seeds.

Expected water content (%)	Number of unviable seeds (%)				Average
	Storage duration (weeks)				
	2	4	6	8	
21–25	0.7	0.0	0.0	0.0	0.18
16–20	0.0	0.0	0.0	0.0	0.00
11–15	0.0	0.0	0.0	0.0	0.00
6–10	0.0	0.0	0.7	0.0	0.18
Average	0.18	0.00	0.18	0.00	
CV* = 14.43 %					

*Coefficient of variance (CV) was obtained after the data transformed with $\sqrt{y+1}$.

Discussions

The high percentage of normal seedlings achieved from this study, particularly in the lowest level of water content indicated that the seeds required minimum water content to accelerate the period of its after ripening dormancy. Bewley and Black (1985) mentioned that environmental factors, such as temperature, humidity and oxygen level affected the length of after ripening period. When seed water content was maintained under minimum state, the possibility of after ripening occurrence could be inhibited. Regarding the process of after ripening breaking, the seeds also favored storage for certain period to encounter this dormancy effect. As stated by Harjadi (1979), freshlyharvested rice seed would be difficult to be germinated directly, thus required an incubation treatment during certain time period.

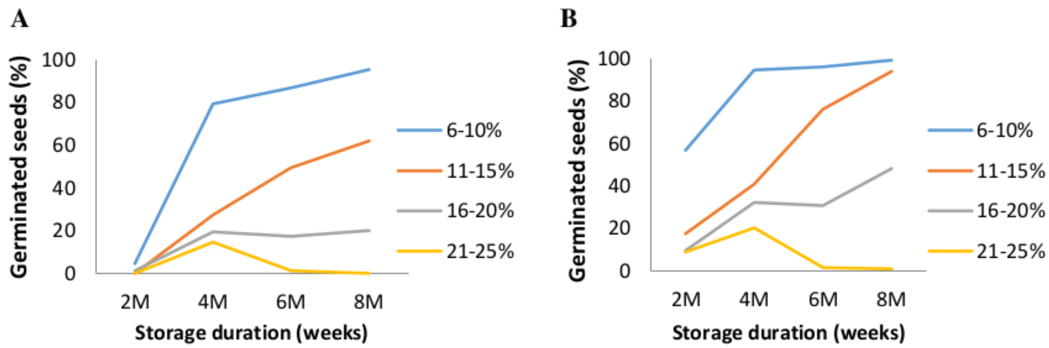

Figure 4. Effect of seed water content and storage duration toward the germination percentage of brown rice seeds on first count (a) and soil emergence (b).

A successful seed germination is marked by the high percentage of normal seedlings. Kamil (1986) described that normal seedling was defined as a seedling that grew with long primary roots complemented with abundant secondary roots. This seedling also showed the presence of permanent root emerged from the first nodus (crown root) and normal first leaf commonly appeared from coleoptile. The number of normal seedlings obtained from an assay indicated the quality of the seed used. For mass application, information regarding the seed quality is crucial to guarantee the cultivation success economically (Ilyas, 2012). Other information regarding the seed vigor would also describe the seed quality and specification required for commercial purpose (Lindayanti, 2006). The utilization of low quality seed would result in abnormal seedling with less uniform growth and high chance of seed-borne pathogen infection.

Table 5. Effect of seed water content and storage duration toward the number of dormant seeds.

Expected water content (%)	Number of dormant seeds (%)			
	Storage duration (weeks)			
	2	4	6	8
21–25	49.33 cA	4.67 cB	1.33 cBC	0.67 cC
16–20	79.33 bA	22.00 bB	3.33 cC	1.33 cC
11–15	92.67 abA	31.33 bB	12.67 bC	6.67 bD
6–10	99.33 aA	100.00 aA	97.33 aA	92.00 aA
CV* = 9.45 %				

Numbers on the same row followed by the same uppercase and numbers on the same column followed by the same lowercase were insignificantly different according to DNMRT with a $p<0.05$.
*Coefficient of variance (CV) was obtained after the data transformed with $\sqrt{y+1}$.

Abnormal seedling could appear if a seed did not obtain sufficient environmental supports, such water, oxygen, temperature or light. This lack of environmental factors would restrict the completion of each metabolism proccess that a seed required to germinate as a normal seedling. A seed had to go through several metabolism processes, such as water absorption, enzymes activation, and nutrients utilization that would trigger the cell division and differentiation on meristematic spots (Sutopo, 2010). The failure occurred in one of these processes would cause the growth abnormality of a seedling characterized by the absence of root and first leaf, the emergence of weak primary root and plumule, the presence of watery or decayed plumule, the presence of colorless coleoptile, and the seed decayed (Kamil, 1986).

Table 6. Effect of seed water content and storage duration toward the number of germinated seeds on first count test.

Expected water content (%)	Number of germinated seeds (%)							
	Storage duration (weeks)							
	2		4		6		8	
21–25	4.67	aB	79.33	aA	86.67	aA	95.33	aA
16–20	0.00	aC	27.33	bB	49.33	bA	62.00	aA
11–15	1.33	aB	19.33	bcA	17.33	cA	20.00	bA
6–10	0.00	aA	14.67	cA	1.33	dA	0.07	cA
CV* = 24.69 %								

Numbers on the same row followed by the same uppercase and numbers on the same column followed by the same lowercase were insignificantly different according to DNMRT with a $p<0.05$.
*Coefficient of variance (CV) was obtained after the data transformed with $\sqrt{y+1}$.

Besides abnormal seedlings, the other indicator of low quality seeds is the presence of unviable seeds. An unviable seed is characterized by the presence of rot symptom found before germinated or it unable to emerge after certain time period but not because of dormant state (Sutopo, 2002). A seed could be unviable due to the damage of cellular tissues leading to seed inability to develop or germinate (Fitri, 2013). The pathogen infection might also cause the unviability of a seed. Seed-borne pathogens infection could occur because of inappropiate seed storage condition. It was commonly triggered by high humidity and water content inside the seed itself. Storaging a seed in a humid place increased the chance of pathogens infection. Since it infected a seed, it was probably undetectable before the seed was successfully germinated. These seed-borne pathogens usually become active by utilizing the nutrients produced throughout the seed metabolism during the germination process (Sutopo, 2010).

Unlike unviable seeds, the dormant seeds is commonly occurred in rice seeds, especially if it is recently harvested. Rice seeds tend to encounter after ripening phase where the seeds unable to germinate eventhough the environmental condition is in an optimum state for the germination. This type of dormancy usually occurred right after rice harvesting and took 0 tp 11 weeks depending on postharvest condition applied for the seed (Sinambela, 2008). The period of this after ripening varied in every rice variety. Ilyas and Diami (2007) reported that four dryland rice varieties displayed different dormancy persistence after stored in room temperature with 81-89 % relative humidity. The shortest dormancy period achieved from *Kalimutu* variety that broke after 3 weeks storage. Variety of *Way Rarem* required 4 weeks to break the after ripening, while *Gajah Mungkur* and *Jatiluhur* took longer storage duration (6 and 9 weeks, respectively).

The breaking of after ripening in rice seeds was more stimulated as the storage duration was prolonged. Bustamam (1989) explained that cereal seeds were unable to germinate right after harvesting. To induce its germination ability, these seeds were recommended to be stored in dry condition at room temperature to break its dormancy. The after ripening was categorized as endogenous physiological dormancy where the dormant state began when ABA content in seeds increased thus suppress the production of gibberelin. Dry storage treatment was aimed to stimulate the production of gibberelin by the seed to achieve the balance state the ABA level, so that the dormancy could be broken and seed could start to germinate.

One of obstacle inhibiting the dormancy breaking of a seed is seed water content. Rice seeds usually contained water up to 30 % at the time of harvesting. This high water content triggered the dormancy to happen. Sadjad (1980) mentioned that the seed germination could occur once seed lost most of its water during the drying treatment. Once the dormancy broken, the germination proccess would start and the seed would obtain its viability and vigor to grow and emerge from the soil. Previous studies reported that number of emerged seedlings from several rice local varieties increased along with the longer storage duration (Ratu, 2011; Fitri, 2013). Additionally, Ilyas (2012) also stated that seed water content required to be maintained at minimum level to prevent seed-borne pathogen infection and maintain the maximum seed quality. The excess water content of a seed could stimulate seed deterioration rapidly due to pathogen activity inside the seed.

Conclusions

The best combination of seed water content and storage duration stimulating higher viability on brown rice seeds of *Daro Merah* was achieved from 6-10 % water content and 8 weeks storage duration. At this condition, the germination resulted 98.67 % normal seedlings with no dormant and unviable seeds. The seed vigor revealed 95.33 % germination on first count test and 99.33 % on soil emergence assay.

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